In order to answer these - at least to some extent - search on MEDLINE for relevant articles on CO & atherosclerosis. - selected the following -

N.B. Zhu et al exposures re CO = 60.2 ppm (high) } 10 weeks.
18.8 ppm (low)

Bowyer & Ford, 1983. (TE1564)

"The effects of carbon monoxide on the coronary arteries of cynomolgus Monkeys - Macaca fascicularis - fed an atherogenic diet".

21 animals 7 = normal diet / air
7 = 6 wks normal diet → atherogenic diet at ++ / air.
7 = " " " " " " / CO, 200 ppm, 7 hr / day.

6 months total expt.

Results - CO group = higher mean plasma cholesterol

"The atherosclerotic disease was significantly increased in severity at the same sites that were affected in Group 2. Therefore CO appears to exacerbate the disease at existing sites rather than initiating new disease sites."

D.R. Allen, N.L. Bowse, D.L. Ruth, L. Butler & C. Fletcher. (1988)

"The effect of cigarette smoke, nicotine, and CO on the permeability of the arterial wall"

J Vasc. Surgery 1988; 7: 139-52.

Dogs: intubated :- control
7 dogs - smoke - 4 cigs / hr, 3 hrs (3 hr expt)
4 dogs Nicotine infusion :- 1 mg/kg/hr 1½ hr (3 hr expt)
4 dogs CO 400 ppm. 2 hrs 8 hrs (3 hr expt)

Basic finding = ↑ permeability in CO & smoke exposed groups only - fairly good correlation between COHb levels & permeability although better for pure CO than for smoke.

Also suggest intimal hypoxia may be playing a role

Hugod, C. (1981)

"Myocardial morphology in rabbits exposed to various gas phase constituents of tobacco smoke."

Atherosclerosis, 40 (1981): 181-190

"The threshold exposure limit for the occurrence of these changes was 4h exposure to 100 ppm CO for 4h."

♂ albino rabbits exposed from 1-7 weeks
= normal diet.

HCN = 0·28 - 0·47 ppm.
CO = 200 - 300 ppm
NO = 3·4 - 4·9 ppm.

No groups showed sig diffs between exposed and controls.

Rogers et al. (1980).

"Atherosclerosis-related Responses to Cigarette smoking in the Baboon".

Circulation 61; No 6 (1980): 1158-

6 ♂ baboons
wks 1-5 = baseline measurements; wk 6 = atherogenic diet.
Cigarette & main smoke:-
by wk 60 - cig every 15 min, 12 hrs/day
4 monkeys smoking ⇒ ↑ lymphocyte counts & fasting blood glucose

---

C Hugod et al (1978).

"Effect of Carbon Monoxide Exposure on Aortic and Coronary Intimal morphology in the Rabbit" A reevaluation

Atherosclerosis, 30 (1978) 333-342.

Non-cholesterol-fed rabbits were exposed to CO at 200 ppm.
low level exposure ≡ 200ppm ½, 2, 4, 6 or 12 weeks.
high level exp. ≡ 2000 ppm 320min
≡ 4000 ppm 205min.

Claim virtually no effects.

Fd & Cosmetic. Toxicol., Vol 12/1. (1974)
"Carbon Monoxide & atherosclerosis"
Review:-
Animals - dogs with exptlly induced M.I. exposed for 23 hrs/day for 14 wks to an atmosphere of 115 mg CO/m³. The average COHb blood levels in exposed animals showed 14% saturation compared with 1.3% in controls breathing air.
No cv changes found due to CO.
Similar lack of toxicity in Monkeys, 15 - 60 ppm CO, 22 hrs/day 7 days/wk - 2 years.

Davies, R.F. et al. (1976).
"The Effect of intermittent CO exposure on experimental atherosclerosis in the Rabbit".
Atherosclerosis 24(1976) 529-536.

Hypercholesterolaemic Rabbits - 2% cholesterol.
≈250 ppm - daily for 10 weeks.
The extent of coronary artery atherosclerosis was statistically significantly higher in the CO group than in the control group.

	CO	Sham.
% Coronary art affected	50.6 ± 2.4	25.5 ± 2.2.
% stenosis.	62.0 ± 2.2	69.6 ± 1.9.

This would suggest that the low level of CO to which the rabbits are exposed in the final study are unlikely to be the full story.

D.M. Turner, (1949).

"Carbon Monoxide, Tobacco Smoking and the Pathogenesis of Atherosclerosis"

Preventive Medicine, 8: 303-309.

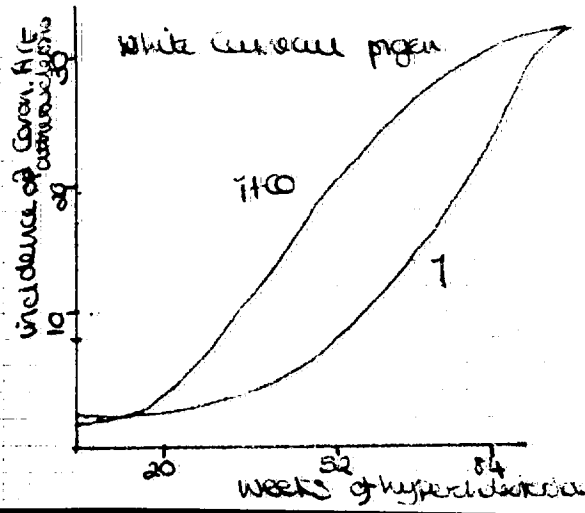
CO ⇒ mean daily COHb ~~increase~~ of 6% in hypercholesterolaemic pigeons ⇒ ↑ coronary artery atherosclerosis.

Time of exposure, extent of hypercholesterolaemia and COHb conc are crucial - too long & ≡ to much, too low or too early and no effect is observed.

Explained by multiphasic model. —

1% cholesterol ↑ disease dupt after big phase & proceeds v rapidly due to ↑ arterial uptake of plasma lipids. Eventually the phase of lipid uptake is reduced due to the presence of a significant number of lesions that may affect the local "critical morphology" & hence membrane transport processes. Increased lipid uptake may also affect membrane transport of lipid in adjacent apparently uninvolved regions. Endogenous processes governing lesion development may then tend to predominate and, because some substrates, including oxygen are limiting, the process will slow down.

CO exposure appears to enhance, by methods which are not yet clear, the initial uptake of plasma lipid and so thus phase of lesion development accelerates. Eventually, however, the "endogenous" lesion dupt starts to predominate and because it is less affected by COHb. the rate of progression eventually becomes similar to that in controls:-



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Back to Rosemary's search !!

ref 11.

Born G.V. (1991).

"Recent evidence for the involvement of catecholamines & of macrophages in atherosclerotic processes". Ann. Med. 1991, 23(5): 569-72.

adrenaline & noradrenaline accelerate atherogenic uptake of low density lipoproteins. -

Ref 28.

Shafi, S., Cusack, N.J. & Born, G.V. (1989).

"Increased uptake of methylated low density lipoproteins induced by noradrenaline in carotid arteries of anaesthetised rabbits".

Proc. R. Soc Lond. 1989. Jan 23 235 (1281) p 289-298

N.B [illegible] "physiological" concentrations.

Ref # 36

Fraser, R et al. (1988).

"Nicotine decreases the porosity of the rat liver sieve: a possible mechanism for hypercholesterolaemia"

Br. J. exp. Pathol, 1988; 69(3): 345-50.

Rats fed [illegible] for 6 wks (≡ to 50-100 cigs/day) ⇒ hypercholesterolaemia

Maybe due to [illegible] identified decrease in porosity of hepatic sinusoidal endothelium (40% [illegible]),

Believe that this decreased hepatic sinusoidal porosity may alter cholesterol [illegible] by increasing the circulation time of chylomicron remnants too large to pass through the [illegible].

This phenomenon may be an [illegible] [illegible] etc......

Ref # 54.

Gillespie, M.N. et al. (1988).

"Exaggerated nicotine-induced norepinephrine release from atherosclerotic rabbit hearts."

[illegible], [illegible]. [illegible] 147-157.

In atherosclerotic rabbits found that nicotine 'released' significantly more norepinephrine [illegible] [illegible] than it did in non-atherosclerotic rabbits.

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suggest diet-induced (2% chol) atherosclerosis is associated with an increase in myocardial -- norepinephrine content and that the augmented pool can be mobilised by nicotine.

From original literature search from Rosemary - see p55 back:-

P. Hadjiisky et al. (1991)
"Modèles expérimentaux d'athérosclérose"
Arch. Mal. Coeur. 1991: 84: 1593-603.

3 classes of arterial lesions:-
- initial atheromatous lipid lesions - most often from diet
  - = diffuse lipidosis with infiltration of lipids in the intima
  - = fibro / lipid plaques etc etc .....
- Practically almost proliferative myocyte lesions - intimal thickening
- Arteriosclerotic medial lesions - fibrosis calcifications etc etc.

March 18th 1993

① Possible additional questions to aim at Wato.....

→ HAB - re article from Loreen on passive smoke exposure. ←

N.B. "Volunteer" to do R&D seminar.

~~Bowsen & Stockwell data on occupational exposure.~~

March 19th 1993

Travel :- HER/IAM Lausanne CISC (AFSC) premeeting. 22
PEM/HER/RIT Lausanne ECMA meeting
24th Patent Seminar :- RDE/ANB.

*Ring Loreen - tell her of plans for EACR meeting in April
*Ring Mayoda re 'John Graham' paper - any further developments

Cauliron building - property care & nothing? - other industries etc. which may be in same areas. - look for allies
- Confined space - Restaurants, Cafés, betting shops, pubs etc.....
- Air / vent engineers:
- All industries with gaseous effluents - furniture industry etc etc
Arve group - alcohol, coffee etc etc.....

{ Loreen - programme - "Biomarkers of Chem Exp" *Jun 16-18*
[IFCC] European Conference:
* FOY1 - for Bowman & Stockwell studies. * ←
→ hold notch!!

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22/03/93 InBifo.

Dr Hausman – overview –

Exposure v. odd – 6/8h H/L = 8, 3, & 30 for T, C, & N.

① We can afford to decide our most approp exposure regime.

② Serum cholesterol levels would be up to 8% the study – not follow diet of the Chx.

Armitage – smoking related diseases

Diet: – InBifo = 05%, 2% in diet ⇒ ↑

300

* Co probably not an important group. *

Drop Normocholesterolemic rabbits – not relevant to Human Hx.

Exposure: 6ASS – what is ideal.

Watanabe Rabbit LDL Under recept. deficient strain.
Does not develope pathological liver lesions:
For time being – mechanistic studies – only later – Not Watanabe.

Who? – InBifo / Köln / CEC – exp. with current equipment.
Alternatives: –

① TNO-CIVO; Dr R Wautersen Have non-CLP experts in Leiden, IVO-TNO
Dr VanderWall.

would need advice on ETS ....

② Prof K Schä – Düsseldorf

③ Prof Rahn Medical Univ Münster
focussed on Human.

④ Dr Ludwig – Bonn – not much info? ....

⑤ Dr Drexel, Boehringer-Mannheim
– expert Watanabe rabbits = no contract research but may give advice.

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Species:- As (~~Ziegler Sprague~~) 2hrs but closer dose range

Diet:- is it essential to use the Ziegler diet -? - No but def preferable.

Exposure:- High. RASS:
High. FSS:
Low RASS:
HH RASS:

} Conclusions - why & what would be a 'real' high dose room-exposed

Should use a High FSS:-

See Ted re ETS/SS studies

See HER re IAQ monitoring studies with WBFO

See p44 re Sci. Am. Article to see

If should extend intervals - how?

Objective to publish data:-

Carrying Study -
carv, low, high, 30/gr, Aorta Pulm Art. } Morphometry - Sudan?
Histology -

Review literature again:-
To answer following Qs:
① - what endpoints are investigated?
② - what vessels looked at?
③ - what 'effects' are measured?
④ - Possible effects of dietary intake?

- Should we look at food intake?
What doses.

Morphometry + BC. analyses

⑤ BC extrapolation of ~~data~~. how has it been used in literature

---

2 alternatives - ie. v. simple No 1 - only high dose.
or slightly expand to see an improvement
A later study would look at ~~mechanistic~~ mechanistic studies.

Start & Date ≈ Beginning 1994.
So at best = 2½ 3 years to final results (pre study, 10 patients = 18 months from beginning to end)

Other exposure activities — check protocols.....
1R4F cigarette — How ISS yields compare between cigarettes.
2R4F — west coast type cigarette

22nd April 1993. Check HSE's availability

In BB to write up protocol — roughly as above
? What impact will this have in several years time —
How can we try and predict other publications in this field in the intervening years? — Check Directory ORSH
? How effective is this response going to be to Glantz & Parmley's paper.....

Dose regimes — eventually decided upon FSS at comparable (or maybe slightly higher) CO levels.

Cotinine - BN = 10% of Nic.
↑ SD ≈ 50% of Nic. - possibly due to contamination from urine.

Possible that SD (& possibly BN) cotinine in hair comes entirely from external contamination.
Still don't know what's happening - could be Melanin but structural group - pure speculation!! - If chem-bound' a changed during extraction. might not free it for analysis:

Re induction - induced is much less than uninduced in both SD & BN. probably due to lack of availability -
No

Hamster study - take up much more than BN. Nat ind ≈ 100 µg/g.
ind ≈ 30 µg/g.

Non-induced hamster - virtually no nicotine in urine after bolus.

Data missing - Blood plasma-studies - should be ≈ 2 months:

Plasma levels only taken at the end.

Equiv to human systemic uptake for 20/day smoker - 0.7 µg/g.
In SS exposure found human & Rat hair took up approx same
∴ could suggest that systemic uptake in humans is very low compared to external
Maybe preferable to compare plasma concs of rat to plasma concs of humans'

Publication - once plasma produced could be ready - would look at Rats. could introduce a discussion regarding the contribution of systemic uptake extrapolated to a human smoking situation.

Sprague Dawley - not sure of internal uptake or external contamination but could say that it is at most very small
BN however has much more uptake & systemic uptake -
underlies importance of pigmentation

Paper should introduce as discussion points:-

1. Pigment makes a big difference re systemic uptake but not external
2. Systemic uptake ~~[illegible]~~ compared to external in rat & if extrapolate to human smoking suggests that

Why SD rats not take-up nic in follicle:-

Pigmented rat hair in nic study: 2µg/ml water ⇒ 50ng nic/g hair.
1µg nic/ml water.

—#—

<u>Conclusions</u>:-

Rat study should be ready for publication as soon as the plasma data is available. The rat study so far shows that there is systemic uptake in Brown Norway rats but not in SD rats which would implicate melanin in the uptake role. Furthermore a comparison of systemic uptake concentrations with concentrations found in ETS exposed rats should show that the systemic contribution will be minimal. How this could be extrapolated to humans is pure speculation and ∴ probably should not be included. However it may be worth putting our speculation in – i.e. that systemic contribution must be very very small. Plasma data should be ready in around 2 months then will prepare a publication.

No need to study further the rat question. It is puzzling that there should be such a big difference between external uptake in SD and internal. The External mechanism should also play a part in the internal role – surely? The results suggest some form of barrier between ~~external uptake & int~~ nicotine in blood and that in hair. ~~[illegible]~~ – Could this be competition from 'skin' – a skin "sink"? – unlikely.

NB. 4-AB discussions w Dr Hausmann-Raw Nitch!!

- 1986 Animal Protection law to walk.....

Future studies:-

Systemic contribution in humans needs to be established as being v. v. low.

1) - could get a better idea using beard hair & looking at the gradient
Recommendation:- do beard gradient study to provide further information that this is justified (systemic is small & 'external' model is justified)

2). which other factors we should look at:-
Bleaching; washing; hair treatment etc etc....
Should use standardised hair sample e.g. wig hair?
Commercially available hair source:-

Summarise:- Missing data on Animals- plasma etc 1 & 2 months.
Replication
Try & find source of commercial hair
Consider exposure of hair..... in approx 2 months time.
Beard study - approx 3 people from inBrio.
Patch study - postponed.....

Think what studies should be investigated in hair

Prepare mini-beard study

Phone call w Tony Andrade

Gutstein / Vesa - stress model - Electrical activity to rat - stress - stress induced atherosclerosis plaques

Stress factor - ADA - } Tony Andrade. Stress - paper
Fax proposal details to ADA - }

Quick call visiting May 11th - 12th - maybe worth discussing
8/14th to Inbrio y/ CRC. possibly w RCA.

2) Vandten: Influence of Ageing & Surface

① ~~surface-contact & ageing - not vice versa - not much details on 'ageing'~~

HSR - p2 - Room-ageing .... still within one day.

HSR - p2 - should specify same conditions - "experimental" room ageing.

HSR - what about desorption - should it be mentioned at all? any data which suggests it might be limited.

HSR p4 - 90 day expts - specify time interval of measurement

p5 - ① specify standardisation for CO - not actual levels.

② wording of last description = misleading.

③ 90 day stability - only TPM shown. refer to other data if known - or show it.

On Table 1. - introduction of each item separately not clear
∴ bracketed explanation.

Pure nic gas [illegible] RJR - [illegible] pure nicotine
. Cambridge filter :- [illegible] particle
phase [illegible] gas phase [illegible]

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MARCH 17th 1993.

COMMENTS from HSE/ROE re Lubrifo publication proposals:-

① Teredesai — Histopathological findings.

Major concern is that the overall message that comes across is that there is a partial effect albeit with provisos.

To reduce the 'impact' would recommend more contextualisation of the FSS exposure stemming vd ETS but putting in some sort of explanation of why this exposure was used - i.e. what was the experiment investigating & why.

Specifically:-

p3. states "The TPM cancs in the FSS of 2 & 6 µg/L are sig above the levels reached in occupied spaces with smoking".

= to me this begs the question why do it then? - what is trying to be modelled, and how much more than normal levels are these?

p5/6. Why not put the findings for the hamsters first to try & emphasise a little more?

p6 - adaptive response - by what?-
"mainly" in the high FSS = of course?!

a dose-dependent increase *

Table 7. HSE - when calculated ratios of high to low exp the TPM is disproportionately low in the high group - any reason - is this significant? - - - - -

Terminal - after 10 weeks - Aorta ⇒ lipid analysis
Heart

Blood withdrawal from 4 animals in each group (non fasted)

Heart weighed & fixed in formol calcium 6 blocks

Morphological studies:

at sac 12 aorta

pulmonary artery

Other & alternative measures of atherosclerosis –

Hawkins and Wigod, 1981:-

Sections of coronary artery
aortic arch
upper thoracic aorta
lower thoracic aorta
abdominal aorta.

} Lipid stains – intimal morphological damage

Fisher et al 1974.

Biochem. Atric blood, time of death, after an overnight fast

- total lipids
- triglycerides
- total cholesterol
- phospholipids
- α_1 & β lipoproteins

+ Coronary angiography

+ aortic atherosclerosis ? – how? "bleeding"

Histopathology – lungs, heart, aorta, arteries, pancreas, spleen, salivary glands & thyroid

L & R coronary arteries – H/E stain

+ fluorine at pH 6, 1: 10,000 → metachromasia

PAS reaction.

Jones et al 1976.

Social studies – Blood samples after overnight fast from marginal ear vein immediately before diet, after 2 weeks feeding, and weekly during exposure

⇒ Plasma cholesterol
triglycerides
CoHb
haemoglobin
plasma glutamate oxaloacetate transaminase.

weighed weekly

could somewhat clinically make a comparable judgment

"Normal" endpoints looked for based on the above methods

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Exposure Monitoring:

In chambers:- CO
TPM
(+RSP)
NIC

? how often

Any other endpoints e.g. Hb etc

Biological endpoints:-

? justify endpoints

? est thus the best timing

Bleeding time:-
circulating platelet aggregates
platelet count
Haematocrit
Hemoglobin
total serum cholesterol
serum retinine
triglyceride levels
HDL cholesterol

} At t=0, 6 & 12

Clark / Parmley - Protocol - SR850 "4 people
SR 1000 with permission
J Jain - 800 SR

Species:

New Zealand, male, 20 - 2.6 kg

Housing: Separately in exposure chambers

Diet: - 3% soybean oil } Ziegler Bros Inc
0.3% cholesterol }

Smoke exposure:- cigarettes - Standard 1st. cigs = exp Marlb? * Should we look at equiv exposure to Marlb - or ? match on CO/1pm/1mic ? What is main advant.

Sidestream smoke - (Presumably fresh SS smoke) from "ETS-H" 4 cigarettes every 15 mins - 6hs/day ? - how would this smoke exposure compare using our methodology w.r.t the levels they reported to measure? - by diff. which would we look to.

"ETS-L" " " " = MS collected through long tube * Surely we should look for a pair test at lower dose? - ie SS - fresh but with higher dilution? - eg and equate to "TPM" dose & to CO on what? -

Exposure groups

① high cholesterol diet for 12 weeks

② high cholesterol diet for 12 weeks
+ ETS-H exposure for 10 weeks (6h/day 5d/wk)

③ high cholesterol diet for 12 weeks
+ ETS-L exposure for 10 weeks (6h/day 5d/wk)

④ high cholesterol diet for 12 weeks
+ CO (at ETS-H level) for 10 wks (6h/d 5d/wk) * Additional controls

⑤ high cholesterol diet for 12 weeks *?
+ Acrolein / Stress inducer

⑥ Normal diet rabbits *?

Additional Info:

COMMENTS:

? do it necessary to use same manufacturer?

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Nicotine in hair
fos/jun and transcip
signal transduction
Rabbit studies
Atherosclerosis studies.

Roses Party

Food

Chocolate cake [- home?]

Carrot sticks tiny sandwiches

Crisps

Pate and toast + cheese

Biscuits

Cheese

Mini rolls

lucky dip

Pass the Parcel

Make crowns

Blind man's bluff

Memory game - x "presents" on tray - remove 1 - see if can remember which it was.

Face paints?

Jenny

Pass the parcel

Make crowns

Play lotto